

AMENDMENTS TO THE CLAIMS

1. (currently amended) An isolated chimeric protein having enzymatic activity of an amadoriase, which chimeric protein comprises, from N-terminus to C-terminus:

a) a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues, wherein the leader sequence comprises the amino acid sequences set forth in SEQ ID NO:1; and

b) a second peptidyl fragment comprising an amadoriase, wherein the amadoriase comprises the amino acid sequence set forth in SEQ ID NO:3.

2-13. (canceled)

14. (original) The isolated chimeric protein of claim 1, wherein the first and second peptidyl fragments are linked via a cleavable linkage.

15. (currently amended) The isolated chimeric protein of claim 1, which further comprises, at its C-terminus, a third peptidyl fragment comprising a second bacterial leader sequence from about 5 to about 30 amino acid residues, wherein the second bacterial leader sequence comprises the amino acid sequence set forth in SEQ ID NO:4.

16-20. (canceled)

21. (original) The isolated chimeric protein of claim 1, which further comprises, at its C-terminus, a third peptidyl fragment comprising a peptide tag.

22. (original) The isolated chimeric protein of claim 21, wherein the peptide tag is selected from the group consisting of FLAG, HA, HA1, c-Myc, 6-His, AU1, EE, T7, 4A6, é, B, gE and Tyl tag.

23. (original) The isolated chimeric protein of claim 15, which further comprises, at its C-terminus, a fourth peptidyl fragment comprising a peptide tag.

24. (original) The isolated chimeric protein of claim 23, wherein the peptide tag is selected from the group consisting of FLAG, HA, HA1, c-Myc, 6-His, AU1, EE, T7, 4A6, é, B, gE and Tyl tag.

25. (original) The isolated chimeric protein of claim 1, which comprises the amino acid sequence set forth in SEQ ID NO:5

(MGGSGDDDDLALAVTKSSSLIVGAGTWGTSTALHLARRGYTNVTVLD
PYPVPSAISAGNDVNKVISSGQYSNNKDEIEVNEILAEAFNGWKNDPLFK
PYYHDTGLLMSACSQEGLDRLGVRVRPGEDPNLVELTRPEQFRKLAPEGV
LQGDFPGWKGYFARSGAGWAHARNALVAAAREAQRMGVKFVTGTPQG
RVVTLIFENNDVKGA VTGDGKIWRAERTFLCAGASAGQFLDFKNQLRPT
AWTLVHIALKPEERALYKNIPVIFNIERGFFFEPEERGEIKICDEHPGYTN
MVQSADGTMMSIPFEKTQIPKEAETVRALLKETMPQLADRPFSFARICW
CADTANREFLIDRHPQYHSLVLGCGASGRGFKYLP SIGNLIVDAMEGKVP
QKIHელიKWNPDIAANRNWRDTLGRFGGPNRVMDFDVKEWTNVQYRDI
SKLKGELEGLPIPNPLLRTGHHHHHH).

26. (withdrawn) An isolated nucleic acid comprising a nucleotide sequence encoding the chimeric protein of claim 1.

27. (withdrawn) An isolated nucleic acid comprising a nucleotide sequence encoding the chimeric protein of claim 25.

28. (withdrawn) The nucleic acid of claim 26, which comprises the nucleotide sequence set forth in SEQ ID NO:6

(ATGGGAGGTTCGGGTGACGATGATGACCTGGCTCTCGCCGTCCTAA
GTCATCATCTCTCCTGATCGTTGGTGCCGGGACTTGGGGCACCTCAAC

GGCTCTGCACCTCGCGCGCCGCGGATATACCAACGTTACCGTGCTGGA
CCCCTATCCTGTCCCTAGCGCCATCTCCGCCGGAACGACGTGAACAA
AGTCATTAGCAGTGGCCAATATTCTGAATAACAAAGACGAAATCGAAG
TGAATGAGATCTTGGCGGAAGAGGCGTTTAACGGTTGGAAGAACGAC
CCGCTTTTCAAACCGTATTATCATGATACGGGCCTGCTGATGTCTGCTT
GCTCGCAGGAGGGCCTGGATCGCCTGGGCGTCCGGGTACGTCCGGGCG
AGGATCCTAATCTGGTGGAACTTACCCGCCCGGAGCAATTTTCGTAAAC
TGGCCCCGGAAGGCGTGTTGCAAGGTGATTTTCCGGGTGGAAGGGT
ACTTTGCGCGTTCCGGCGCTGGCTGGGCACATGCAAGGAATGCCTTAG
TGGCAGCAGCACGGAAGCACAGCGCATGGGTGTAAAATTTGTACTG
GCACCCCGCAGGGTCGTGTAGTCACGTTAATCTTTGAAAATAACGATG
TAAAAGGTGCCGTTACGGGCGATGGCAAATTTGGAGAGCGGAACGT
ACATTCCTGTGTGCTGGGGCTAGCGCGGGTCAGTTCCTAGATTTCAAG
AATCAACTTCGACCAACCGCTTGGACCCTGGTACACATTGCGTTAAAA
CCGGAAGAACGTGCGTTGTACAAAAATATACCGGTTATCTTTAACATC
GAACGGGGGTTTTTCTTTGAACCCGATGAGGAGCGCGGTGAGATTAAA
ATATGCGATGAACACCCGGGCTACACAAATATGGTCCAGAGTGCAGA
CGGCACGATGATGAGCATTCCGTTGAAAAAACCCAGATTCCAAAAG
AAGCCGAAACGCGCGTTCCGGGCCCTGCTGAAAGAGACAATGCCCCAG
CTGGCAGACCGTCCATTTCAGCTTCGCACGCATTTGCTGGTGTGCCGAT
ACCGCGAATCGCGAATTCCTGATAGATCGACATCCGCAGTACCACAGT
CTTGTGTTGGGCTGTGGTGCGAGCGGAAGAGGGTTTAAATATCTGCCT
TCTATTGGGAATCTCATTGTTGACGCGATGGAAGGTAAAGTGCCGCAA
AAAATTCACGAATTAATCAAGTGGAACCCGGACATTGCGGCGAACCGT
AACTGGCGTGATACTCTGGGGCGTTTTTGGCGGTCCAAATCGTGTGATG
GATTTTCATGATGTGAAGGAATGGACCAATGTTTCAGTATCGTGATATT
TCCAAGCTGAAAGGAGAGTTGGAAGGTaaGCCAATCCCTAACCCGTTA
CTGCGCACAGGCCATCACCATCATCATCATTA).

29. (withdrawn) An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of claim 26.

30. (withdrawn) A recombinant cell containing the nucleic acid of claim 26.

31. (withdrawn) A method of producing a chimeric protein comprising growing a recombinant cell containing the nucleic acid of claim 26 such that the encoded chimeric protein is expressed by the cell, and recovering the expressed chimeric protein.

32. (currently amended) ~~The product of the method of claim 31~~ A chimeric protein produced by growing a recombinant cell containing a nucleic acid comprising a nucleotide sequence encoding the chimeric protein of claim 1 such that the encoded chimeric protein is expressed by the cell, and recovering the expressed chimeric protein.

33. (original) A method for assaying for a glycosylated protein in a sample, which method comprises:

a) contacting a sample to be assayed with a protease to generate a glycosylated peptide or a glycosylated amino acid from a glycosylated protein, if contained in said sample;

b) contacting said generated glycosylated peptide or glycosylated amino acid with a chimeric protein of claim 1 to oxidize said glycosylated peptide or glycosylated amino acid; and

c) assessing oxidation of said glycosylated peptide or glycosylated amino acid by said chimeric protein to determine the presence and/or amount of said glycosylated protein in said sample.

34. (original) The method of claim 33, wherein the sample is a blood sample.

35. (original) The method of claim 34, wherein the blood sample is a plasma, serum, red blood cell or whole blood sample.

36. (original) The method of claim 33, wherein the glycosylated protein to be assayed is glycoalbumin or glycohemoglobin.

37. (original) The method of claim 33, wherein the protease is an endo-type protease or an exo-type protease.

38. (original) The method of claim 37, wherein the endo-type protease is selected from the group consisting of trypsin, α -chymotrypsin, subtilisin, proteinase K, papain, cathepsin B, pepsin, thermolysin, protease XVII, protease XXI, lysylendopeptidase, prolether and bromelain F.

39. (original) The method of claim 37, wherein the exo-type protease is an aminopeptidase or a carboxypeptidase.

40. (original) The method of claim 33, wherein the protease is selected from the group consisting of proteinase K, pronase E, ananase, thermolysin, subtilisin and cow pancreas proteases.

41. (original) The method of claim 33, wherein the protease generates a glycosylated peptide from about 2 to about 30 amino acid residues.

42. (original) The method of claim 33, wherein the protease generates glycosylated glycine, glycosylated valine or glycosylated lysine residue or a glycosylated peptide comprising glycosylated glycine, glycosylated valine or glycosylated lysine residue.

43. (original) The method of claim 33, wherein the chimeric protein comprises the amino acid sequence set forth in SEQ ID NO:5.

44. (original) The method of claim 33, wherein the chimeric protein is encoded by the nucleotide sequence set forth in SEQ ID NO:6.

45. (currently amended) The method of claim 33, wherein the oxidation of the glycosylated peptide or glycosylated amino acid is assessed by assessing consumption of the glycosylated peptide or glycosylated amino acid, $[[H_2O]]$ or O_2 in the oxidation reaction or the formation of the oxidized glucose (glucosone), H_2O_2 or the amino acid in the oxidation reaction.

46. (original) The method of claim 45, wherein the O₂ consumption is assessed by an oxygen electrode.

47. (original) The method of claim 45, wherein the H₂O₂ formation is assessed by a peroxidase.

48. (original) The method of claim 47, wherein the peroxidase is horseradish peroxidase.

49. (original) The method of claim 47, wherein the H₂O₂ formation is assessed by a peroxidase and Trinder reaction.

50. (original) The method of claim 47, wherein the glycated peptide or glycated amino acid is contacted with the chimeric protein and the peroxidase sequentially or simultaneously.

51. (original) The method of claim 45, wherein the glucosone formation is assessed by a glucose oxidase.

52. (original) The method of claim 45, wherein the glucosone formation is assessed by a combination of glucose 6-phosphate dehydrogenase and hexokinase.

53. (original) The method of claim 33, wherein the protease is inactivated before or current with the contact between the glycated peptide or glycated amino acid and the chimeric protein.

54. (currently amended) The method of claim 53, wherein the protease is inactivated by a heat treatment or an inhibitor of the protease, or by a heat treatment if the protease is inactivated before the contact between the glycated peptide or glycated amino acid and the chimeric protein.

55. (original) The method of claim 33, wherein ascorbate interference is countered using a copper (II) compound, a cholic acid or a bathophenanthroline disulphonic acid or a mixture thereof.

56. (original) The method of claim 33, wherein bilirubin interference is countered using a ferrocyanide salt.

57. (original) The method of claim 33, which is used in the prognosis or diagnosis of a disease or disorder.

58. (original) The method of claim 57, wherein the disease or disorder is diabetes.

59. (original) A kit for assaying for a glycated protein in a sample, which kit comprises:
a) a protease to generate glycated peptide or glycated amino acid from a glycated protein, if contained in a sample;
b) a chimeric protein of claim 1 to oxidize said glycated peptide or glycated amino acid; and
c) means for assessing oxidation of said glycated peptide or glycated amino acid by said chimeric protein to determine the presence and/or amount of said glycated protein in said sample.

60. (original) The kit of claim 59, wherein the means for assessing oxidation of said glycated peptide or glycated amino acid by said chimeric protein comprises peroxidase.

61. (original) The kit of claim 60, wherein the chimeric protein and the peroxidase are formulated in a single composition.

62-86. (canceled)